Plasma Cholesteryl Ester Transfer, But Not Cholesterol Esterification, Is Related to Lipoprotein-Associated Phospholipase A(2)

Dullaart, Robin P. F.; Constantinides, Alexander; Perton, Frank G.; van Leeuwen, Jeroen J. J.; van Pelt, Joost L.; de Vries, Rindert; van Tol, Arie

Published in:
Journal of Clinical Endocrinology and Metabolism

DOI:
10.1210/jc.2010-2139

Publication date:
2011

Plasma Cholesteryl Ester Transfer, But Not Cholesterol Esterification, Is Related to Lipoprotein-Associated Phospholipase A_2: Possible Contribution to an Atherogenic Lipoprotein Profile

Robin P. F. Dullaart, Alexander Constantinides, Frank G. Perton, Jeroen J. J. van Leeuwen, Joost L. van Pelt, Rindert de Vries, and Arie van Tol

Department of Endocrinology (R.P.F.D., A.C., F.G.P., R.d.V., A.v.T.) and Laboratory Center (F.G.P., J.J.J.v.L., J.L.v.P.), University of Groningen and University Medical Center Groningen, 9700 RB Groningen, The Netherlands; and Department of Cell Biology and Genetics (A.v.T.), Erasmus University Medical Center, 3015 CE Rotterdam, The Netherlands

Context: Plasma lipoprotein-associated phospholipase A_2 (Lp-PLA_2) predicts incident cardiovascular disease and is associated preferentially with negatively charged apolipoprotein B-containing lipoproteins. The plasma cholesteryl ester transfer (CET) process, which contributes to low high-density lipoprotein cholesterol and small, dense low-density lipoproteins, is affected by the composition and concentration of apolipoprotein B-containing cholesteryl ester acceptor lipoproteins.

Objective: We tested relationships of CET with Lp-PLA_2 in subjects with and without metabolic syndrome (MetS).

Design and Setting: In 68 subjects with MetS and 74 subjects without MetS, plasma Lp-PLA_2 mass, cholesterol esterification (EST), lecithin:cholesterol acyltransferase (LCAT) activity level, CET, CET protein (CETP) mass, and lipoproteins were measured.

Results: EST, LCAT activity, CET ($P < 0.001$ for all), and CETP ($P = 0.030$) were increased, and Lp-PLA_2 was decreased ($P = 0.043$) in MetS. CET was correlated positively with Lp-PLA_2 in subjects with and without MetS ($P < 0.05$ for both). EST and LCAT activity were unrelated to Lp-PLA_2, despite a positive correlation between EST and CET ($P < 0.001$). After controlling for age, sex, and diabetes status, CET was determined by Lp-PLA_2 in the whole group ($\beta = 0.245$; $P < 0.001$), and in subjects with ($\beta = 0.304$; $P = 0.001$) and without MetS ($\beta = 0.244$; $P = 0.006$) separately, independently of triglycerides and CETP.

Conclusions: Plasma CET is related to Lp-PLA_2 in subjects with and without MetS. The process of CET, but not EST, may be influenced by Lp-PLA_2. These findings provide a rationale to evaluate whether maneuvers that inhibit Lp-PLA_2 will reduce CET, and vice versa to document effects of CETP inhibition on Lp-PLA_2. (*J Clin Endocrinol Metab* **96: 1077–1084, 2011)**

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2; platelet-activating factor acetylhydrolase) is a calcium-independent phospholipase that is secreted by macrophages and other inflammatory cells in the vessel wall (1–4). In human plasma, Lp-PLA_2 is bound to apolipoprotein (apo) B-containing lipoproteins and to some extent also to high-density lipoprotein (HDL) (1–4). Well-characterized activities of Lp-PLA_2 include its ability to hydrolyze low-density lipoprotein (LDL)-derived oxidized phospholipids and long acyl chain phospholipid hy-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-2139 Received September 10, 2010. Accepted December 15, 2010.
First Published Online January 20, 2011

Abbreviations: apo, Apolipoprotein; AU, arbitrary units; BMI, body mass index; CET, cholesteryl ester transfer; CETP, CET protein; EST, cholesterol esterification; HDL, high-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A_2; MetS, metabolic syndrome.

droperoxides, which results in the generation of proinflammatory oxidized nonesterified fatty acids, fatty acid hydroperoxides, and lysophospholipids (1–3, 5, 6). The potential pathogenic role of Lp-PLA_2 in cardiovascular disease development has received much attention in the past few years (1–3). A recent meta-analysis, comprising previously published and unpublished data from more than 79,000 individuals, has unequivocally demonstrated independent positive relationships between plasma Lp-PLA_2 mass and activity levels and incident cardiovascular disease (7).

Despite growing interest in the role of Lp-PLA_2 in atherogenesis, its possible effects on lipoprotein metabolism are largely unknown. The process of cholesteryl ester transfer (CET), mediated by CET protein (CETP), represents an important pathway whereby cholesteryl esters are transported from HDL toward apo B-containing lipoproteins (8–12). Plasma CET will decrease cholesterol in HDL particles and increase the cholesterol content in apo B-containing lipoproteins, as well as contribute to the formation of small, dense LDL. This provides a rationale for the development of novel drugs that inhibit CETP (11–14). Importantly, the rate of CET toward apo B-containing acceptor lipoproteins is affected by their size, protein content, and lipid composition (8, 9, 15–17). In this regard, it is relevant that the CET process is also governed by the charge characteristics of the acceptor lipoproteins, in such a way that negatively charged particles preferentially accept cholesteryl esters (16, 18). This may explain why CET is enhanced upon association of nonesterified fatty acids with cholesteryl ester acceptor lipoproteins (19). In turn, several reports indicate that Lp-PLA_2 associates preferentially with negatively charged apo B-lipoproteins, including small, dense LDL (3, 20, 21). Therefore, it is plausible to hypothesize that the plasma Lp-PLA_2 level is a determinant of CET.

The present study was carried out to determine whether plasma CET is related to Lp-PLA_2, independently of plasma lipoproteins. Enhanced CET has been shown in subjects with the metabolic syndrome (MetS) (22) and accordingly in insulin-resistant individuals (23). We therefore decided to study relationships of plasma CET with Lp-PLA_2 in subjects with and without MetS. Second, because plasma CET and cholesterol esterification (EST) are thought to be coordinately regulated processes (24–26), we also evaluated relationships of plasma EST with Lp-PLA_2.

Subjects and Methods

The protocol was approved by the medical ethics committee of the University Medical Center Groningen, The Netherlands. The participants (aged >18 yr) were recruited by advertisement in local newspapers and provided written informed consent. Physical examination did not reveal pulmonary or cardiac abnormalities. Clinically manifest cardiovascular disease, renal insufficiency, thyroid disorders, liver diseases, current pregnancy, smoking, consumption of more than three alcoholic drinks per day, and statin therapy were exclusion criteria. The use of antihypertensive drugs was allowed. Subjects with type 2 diabetes mellitus (previously diagnosed by primary care physicians using glucose cutoff values as defined by the World Health Organization) were not excluded, except when using insulin or thiazolidinediones.

Body mass index (BMI) was calculated as weight divided by height squared (in kilograms/meter^2). Waist circumference was measured between the 10th rib and the iliac crests. Systolic and diastolic blood pressure was measured in a sitting position with a sphygmomanometer after a 15-min rest. All subjects were studied after an overnight fast.

MetS was defined according to the revised National Cholesterol Education Program Adult Treatment Panel III criteria (27). Three or more of the following criteria were required for categorization of subjects with MetS: waist circumference larger than 102 cm for men and larger than 88 cm for women; hypertension (blood pressure ≥130/85 mm Hg or use of antihypertensive drugs); fasting plasma triglycerides of at least 1.7 mmol/liter; HDL cholesterol below 1.0 mmol/liter for men and below 1.3 mmol/liter for women; and fasting glucose of at least 5.6 mmol/liter or known diabetes.

Laboratory analyses

Venous blood samples were collected into EDTA-containing tubes (1.5 mg/ml). Samples were prepared by centrifugation at 1400 × *g* for 15 min at 4 C. Glucose was measured shortly after blood collection. Samples for other assays were kept frozen at −80 C until assay.

Cholesterol and triglycerides were assayed by routine enzymatic methods (Roche/Hitachi catalog nos. 11875540 and 11876023, respectively; Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured with a homogeneous enzymatic colorimetric test (Roche/Hitachi catalog no. 04713214; Roche Diagnostics GmbH). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Apo B was assayed by immunoturbidimetry (Roche/Cobas Integra Tinaquant catalog no. 030332574; Roche Diagnostics GmbH).

Plasma CET was assayed by a radioisotope method as described previously (26, 28). In short, [^{3}H]cholesterol was equilibrated for 24 h with plasma cholesterol at 4 C, followed by incubation at 37 C for 3 h. Apo B-containing lipoproteins were then precipitated by the addition of phosphotungstate/$MgCl_2$. Lipids were extracted from the precipitate, and the labeled cholesteryl esters were separated from labeled unesterified cholesterol on silica columns. This isotope method is an accurate measure of net mass transfer of cholesteryl esters from HDL to apo B-containing lipoproteins (29). Plasma EST in total plasma was measured as the generation of cholesteryl esters after addition of [^{3}H]cholesterol to plasma according to a previously described procedure (26, 28). EST was assayed using the same incubation system as for the CET assay. The EST rate is linear with time for 5 h, indicating an excess of unesterified cholesterol in the assay system. All assays were performed in duplicate. The CET and EST measurements are expressed in nanomoles per milliliter per

hour. The within-assay coefficients of variation of plasma CET and EST are less than 7.5%.

Plasma CETP mass was measured with a double-antibody sandwich ELISA (courtesy of Dr. G. M. Dallinga-Thie, Amsterdam, The Netherlands) (30). A combination of monoclonal antibodies TP1 and TP2 was employed as coating antibodies, and monoclonal antibody TP20, labeled with digoxigenine, was the secondary antibody. The CETP control samples were validated using a RIA (carried out by Dr. R. M. McPherson, Montreal, Canada). The plasma CETP concentration is closely correlated with CETP activity level measured using an excess exogenous substrate assay (31). Plasma lecithin:cholesterol acyltransferase (LCAT) activity level was determined using excess exogenous substrate containing [^{3}H]cholesterol (32–34). In brief, plasma samples were incubated with labeled substrate for 6 h at 37 C. The reaction was stopped by addition of cold ethanol to the incubation medium. Free and esterified cholesterol were separated using silica columns. [^{3}H]cholesteryl esters were eluted with hexane. In this assay, LCAT activity varies linearly with the amount of plasma in the incubations. LCAT activity measured using a comparable exogenous substrate method has been shown to be strongly correlated with its concentration in plasma (35, 36). LCAT activity was related to the activity measured in human pool plasma and was expressed in arbitrary units (AU; corresponding to the percentage of the activity in human pool plasma; 100 AU is equivalent to 87 nmol cholesterol esterified per ml of plasma per hour). CETP mass and LCAT activity were measured in duplicate, and their intraassay coefficients of variation are less than 5.0%.

Lp-PLA$_2$ mass was assayed using a commercially turbidimetric immunoassay (PLAC Test, diaDexus catalog no. 10-0112; diaDexus Inc., San Francisco, CA) on the Modular P. The manufacturer's instructions for thawing samples were strictly followed. In samples with a measured concentration exceeding 360 μg/liter, the presence of interfering heterophilic antibodies was excluded. The intraassay coefficient of variation was 1.7%, using the EP5 protocol (Evaluation of Precision Performance of Quantitative Measurement Methods) and buffered quality control at a concentration of 150 and 360 μg/liter.

Glucose was measured with an APEC glucose analyzer (APEC Inc., Danvers, MA).

Statistical analysis

Data are given as mean ± SD or as median (interquartile range) and were compared by unpaired *t* tests. Because of skewed distribution, logarithmically transformed values for triglycerides were used. Differences in proportions of variables were determined by χ^2 analysis. Univariate correlations were calculated using linear regression analysis using Pearson's correlation coefficients. Multiple linear regression analysis was performed to disclose independent contributions of variables. Interactions were assessed in additional analyses. To this end, the group mean value of the continuous variable of interest was subtracted from the measured value to obtain a distribution centered on the mean. Product terms between the variables of interest were then calculated. Two-sided *P* values <0.05 were considered significant. For interaction terms, the level of significance was taken at $P < 0.10$.

Results

The study population consisted of 142 Caucasian subjects, of whom 68 fulfilled the criteria for MetS (Table 1). Subjects with MetS tended to be older, but sex distribution was not different between subjects with and without MetS. Twenty-five of the MetS subjects (37%) and five of the subjects without MetS (7%) were on antihypertensive medication ($P < 0.001$) (mostly angiotensin-converting enzyme inhibitors, angiotensin II antagonists, diuretics, and beta-blockers). Type 2 diabetes mellitus was more

TABLE 1. Clinical characteristics, glucose, plasma lipids and lipoproteins, EST, CET, LCAT activity level, CETP concentration, and Lp-PLA$_2$ in 68 subjects with and 74 subjects without MetS

	MetS present (n = 68)	MetS absent (n = 74)	*P* value	*P* value adjusted for age and sex
Age (yr)	58 ± 9	55 ± 10	0.051	
Sex (M/F)	37/31	42/32	0.91	
Type 2 diabetes mellitus (n)	52	23	<0.001	
Systolic blood pressure (mm Hg)	145 ± 19	132 ± 21	<0.001	0.001
Diastolic blood pressure (mm Hg)	89 ± 9	81 ± 10	<0.001	<0.001
BMI (kg/m^2)	29.8 ± 4.4	25.1 ± 3.3	<0.001	<0.001
Waist circumference (cm)	104 ± 13	86 ± 11	<0.001	<0.001
Glucose (mmol/liter)	8.6 ± 2.6	6.2 ± 1.5	<0.001	<0.001
Total cholesterol (mmol/liter)	5.59 ± 1.02	5.49 ± 0.92	0.54	0.58
Non-HDL cholesterol (mmol/liter)	4.40 ± 1.02	3.95 ± 0.96	0.008	0.007
HDL cholesterol (mmol/liter)	1.19 ± 0.34	1.54 ± 0.38	<0.001	<0.001
Triglycerides (mmol/liter)	1.94 (1.66–2.49)	1.12 (0.79–1.46)	<0.001	<0.001
Apo B (g/liter)	1.00 ± 0.23	0.89 ± 0.21	0.004	0.004
EST (nmol/ml · h)	66.8 ± 17.2	52.6 ± 13.9	<0.001	<0.001
CET (nmol/ml · h)	25.7 ± 8.6	19.3 ± 6.3	<0.001	<0.001
CETP mass (mg/liter)	2.52 ± 0.94	2.21 ± 0.70	0.030	0.034
LCAT activity (AU)	117 ± 15	105 ± 15	<0.001	<0.001
Lp-PLA$_2$ (μg/liter)	290 ± 65	313 ± 73	0.048	0.043

Data are expressed as mean ± SD or median (interquartile range). M, Male; F, female.

TABLE 2. Univariate correlations of the plasma Lp-PLA$_2$ concentration with plasma lipids and lipoproteins, LCAT activity level, and CETP mass in all subjects combined (n = 142) and in subjects with (n = 68) and without MetS (n = 74)

	Lp-PLA$_2$		
	All subjects (n = 142)	**MetS present (n = 68)**	**MetS absent (n = 74)**
Total cholesterol	0.294[b]	0.204[a]	0.404[b]
Non-HDL cholesterol	0.292[b]	0.226[a]	0.445[b]
HDL cholesterol	−0.030	−0.070	−0.151
Triglycerides	−0.046	0.000	0.089
LCAT activity	−0.043	−0.109	0.13
CETP mass	0.064	0.007	0.203[a]

Pearson's correlation coefficients are shown.

[a] $P < 0.10$; [b] $P \leq 0.001$.

prevalent among subjects with MetS (76%) compared with subjects without MetS (31%; $P < 0.001$; Table 1). Oral hypoglycemic drugs (sulfonylurea and metformin, either alone or in combination; other hypoglycemic drugs were not used) were used by 39 (57%) of the subjects with MetS and 15 (20%) of the subjects without MetS ($P < 0.001$).

As shown in Table 1, blood pressure, BMI, waist circumference, and plasma glucose were higher in subjects with MetS than in subjects without MetS. Triglycerides, non-HDL cholesterol, and apo B levels were also increased, whereas HDL was decreased in MetS subjects. All these differences remained significant after age and sex adjustment. Plasma EST and CET were increased in MetS subjects, coinciding with higher LCAT activity levels and CETP mass (Table 1). Plasma Lp-PLA$_2$ concentration was on average 7% lower in MetS subjects, and the difference with subjects without MetS remained after controlling for age and sex. However, this difference was lost after additional adjustment for diabetes status ($P = 0.93$).

In all subjects combined, the plasma Lp-PLA$_2$ concentration was correlated positively with total cholesterol and non-HDL cholesterol, but not significantly with HDL cholesterol, triglycerides, LCAT activity levels, and CETP mass (Table 2). Comparable correlations were observed in subjects with and without MetS separately (Table 2), as well as in subjects without diabetes (data not shown).

The univariate correlations of EST with LCAT activity levels, lipids, lipoproteins, and plasma Lp-PLA$_2$ mass are shown in Table 3. Plasma EST was correlated positively with CET in all subjects combined, as well as in the separate groups of subjects with and without MetS. In all subjects together, in subjects with and without MetS separately (Table 3), and in nondiabetic subjects only (n = 67, data not shown), plasma EST was correlated positively with LCAT activity, triglycerides, and non-HDL cholesterol, but no relationship with Lp-PLA$_2$ was observed. In multiple linear regression analysis, plasma EST was found to be predicted independently and positively by plasma triglycerides ($\beta = 0.444$; $P < 0.001$) and LCAT activity levels ($\beta = 0.341$; $P < 0.001$) after controlling for age ($\beta = 0.001$; $P = 0.99$), sex ($\beta = -0.084$; $P = 0.143$), and diabetes status ($\beta = 0.137$; $P = 0.035$). This analysis again did not show a significant contribution of the Lp-PLA$_2$ concentration to plasma EST ($\beta = 0.037$; $P = 0.55$). Further analysis demonstrated that the absence of a relationship of Lp-PLA$_2$ with EST ($\beta = 0.013$; $P = 0.83$) was not confounded by the use of antihypertensive and glucose-lowering drugs (data not shown). Additional multiple linear regression analyses in subjects with and without MetS separately also revealed no independent positive relationship of plasma EST with Lp-PLA$_2$ ($\beta = 0.114$, $P = 0.276$; and $\beta = -0.091$, $P = 0.310$, respectively, data not shown).

Plasma CET was found to be correlated positively with triglycerides and non-HDL cholesterol in all subjects together and in subjects with and without MetS separately

TABLE 3. Univariate correlations of plasma EST and CET with LCAT activity level, CETP mass, triglycerides, non-HDL cholesterol and plasma Lp-PLA$_2$ concentration in all subjects combined (n = 142) and in subjects with (n = 68) and without MetS (n = 74)

	All subjects (n = 142)		**MetS present (n = 68)**		**MetS absent (n = 74)**	
	EST	**CET**	**EST**	**CET**	**EST**	**CET**
CET	0.664[c]		0.521[c]		0.718[c]	
LCAT activity	0.673[c]		0.529[c]		0.722[c]	
CETP mass		0.266[c]		0.321[b]		0.053
Triglycerides	0.695[c]	0.765[c]	0.596[c]	0.700[c]	0.648[c]	0.736[c]
Non-HDL cholesterol	0.432[c]	0.649[c]	0.361[b]	0.613[c]	0.412[c]	0.654[c]
Lp-PLA$_2$	−0.057	0.169[a]	0.010	0.246[a]	0.019	0.240[a]

Pearson's correlation coefficients are shown.

[a] $P < 0.05$; [b] $P \leq 0.01$; [c] $P \leq 0.001$.

TABLE 4. Multiple linear regression analyses showing relationships of plasma CET with CETP mass, triglycerides, and Lp-PLA_2 in all subjects combined (n = 142), subjects with MetS (n = 68), and subjects without MetS (n = 74)

	All subjects		Subjects with MetS		Subjects without MetS	
	β	*P* value	β	*P* value	β	*P* value
Diabetes (yes/no)	0.178	0.003	0.143	0.112	0.220	0.014
Ln triglycerides	0.723	<0.001	0.666	<0.001	0.743	<0.001
CETP mass	0.148	0.005	0.265	0.002	0.015	0.844
Lp-PLA_2	0.245	<0.001	0.304	0.001	0.244	0.006

All models are adjusted for age and sex. β , Standardized regression coefficient.

(Table 3). In all subjects together and in subjects with MetS, plasma CET was also positively correlated with CETP mass. In the combined subjects and subjects with and without MetS separately, plasma CET was correlated positively with Lp-PLA_2 (Table 3). A comparable trend was found in nondiabetic subjects only (n = 67; r = 0.187; $P = 0.129$). Multiple linear regression analysis demonstrated that in the combined subjects plasma CET was independently and positively related to plasma triglycerides, CETP, and Lp-PLA_2 after controlling for age, sex, and diabetes status (Table 4). A graphical presentation of the relationships of plasma CET with Lp-LPA_2 and triglycerides, and of CET with Lp-LPA_2 and CETP mass is provided in Figs. 1 and 2, respectively. Additional multiple linear regression analyses in subjects with and without MetS (Table 4), as well as in subjects without diabetes (β = 0.213; $P = 0.025$), showed similar independent relationships of plasma CET with Lp-PLA_2. The relationship of CET with Lp-PLA_2 remained significant ($\beta = 0.167$; P = 0.003) after further adjustment for non-HDL cholesterol and apo B, as well as after controlling for the use of medication (antihypertensives, sulfonylurea, and metformin) ($\beta = 0.236$; $P < 0.001$; data not shown). No interactions of plasma Lp-PLA_2 with either triglycerides ($\beta = 0.019$; $P = 0.70$) or CETP mass ($\beta = 0.001$; $P = 0.99$) on plasma CET were observed.

Discussion

This study shows for the first time that plasma CET is correlated positively with the Lp-PLA_2 concentration in subjects with and without MetS. This effect could not be ascribed to a relationship between plasma LP-PLA_2 and CETP mass. Furthermore, multiple linear regression analysis revealed that this relationship remained present after adjustment for age, sex, and diabetes status as well as for triglycerides and apo B-containing lipoproteins, and was independent of plasma CETP mass. These results agree with the hypothesis that the process of CET is influenced by

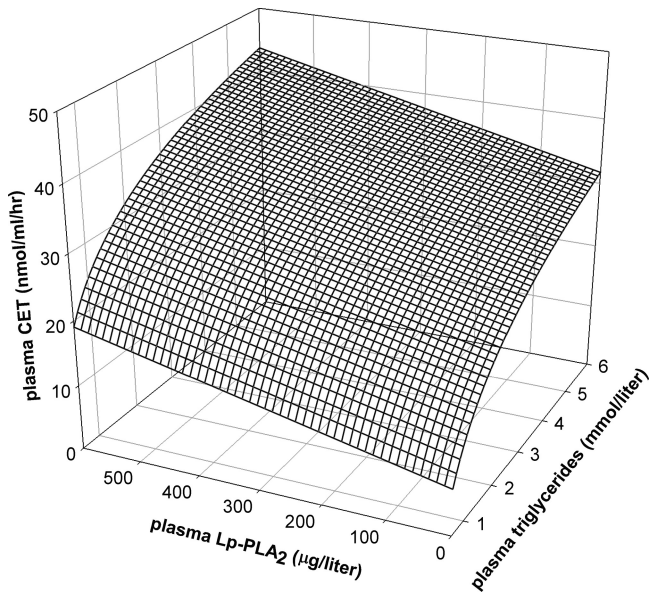

FIG. 1. Graphical presentation of the relationships of plasma CET with Lp-LPA_2 and triglycerides. Standardized regression coefficients (β values) from the multiple linear regression analysis model given in Table 4 are used.

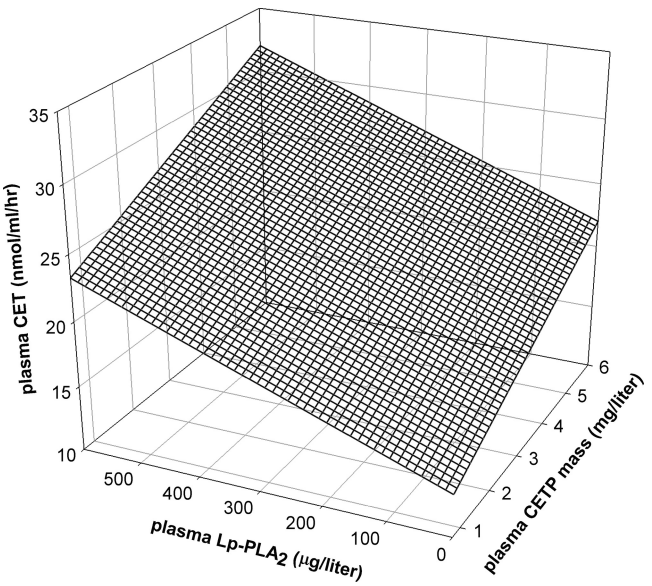

FIG. 2. Graphical presentation of the relationships of plasma CET with Lp-LPA_2 and CETP mass. Standardized regression coefficients (β values) from the multiple linear regression analysis model given in Table 4 are used.

Lp-PLA$_2$. In contrast, plasma EST was unrelated to Lp-PLA$_2$, despite strong interrelations between EST and CET.

We decided to compare subjects with and without MetS in the expectation that plasma CET is increased in MetS (22, 23), as confirmed in this report. Plasma CET was not only affected by the triglyceride concentration, as a reflection of the concentration of triglyceride-rich lipoproteins, but to some extent also by CETP mass. Notably, the effect of CETP mass on CET was not significant among subjects without MetS. This finding is consistent with the proposition that CETP may become rate limiting in the CET process in hypertriglyceridemia (37). In agreement, it was found earlier that plasma CET is only weakly related to CETP activity, measured using an exogenous substrate assay that is closely correlated with CETP mass (25). Likewise, in subjects without diabetes, no independent contribution of plasma CETP mass on CET was observed in another report (17).

Of further relevance, our study showed a moderately decreased plasma Lp-PLA$_2$ concentration in MetS subjects. Increased Lp-PLA$_2$ concentration and activity levels were found previously in nondiabetic subjects with MetS (38), and an increased Lp-PLA$_2$ concentration was found in diabetic patients with MetS compared with those without MetS (39). It is well established that there is sufficient agreement between the concentration and the activity level of Lp-PLA$_2$ in plasma (7). Nonetheless, it has been shown that MetS exerts greater effects on the Lp-PLA$_2$ activity level compared with its plasma concentration (38). Of further interest, the recent meta-analysis revealed lower plasma Lp-PLA$_2$ mass but unaltered activity in diabetes mellitus (7). In line with a decreasing effect of the diabetic state in Lp-PLA$_2$, the difference in its concentration between subjects with and without MetS was not significant after controlling for diabetes status. Thus, the apparent discrepancy with respect to Lp-PLA$_2$ concentration with other reports (38, 39) could be explained at least in part by the considerable number of diabetic subjects in our study population. Although measurement of Lp-PLA$_2$ activity could have provided additional insight in its relation with CET, multiple linear regression analysis did reveal that the plasma Lp-PLA$_2$ concentration contributed to CET independently of diabetes status. Furthermore, comparable relationships of CET with the Lp-PLA$_2$ concentration were seen in the separate groups of subjects with and without MetS as well as in nondiabetic subjects only. Therefore, the conclusion is allowed that the positive and independent relation of CET with the Lp-PLA$_2$ concentration is not confined to subjects with MetS and is not confounded by the presence of type 2 diabetes mellitus.

An increasing body of evidence supports the notion that nonesterified fatty acids and lipid peroxides are accumulated in electronegative LDL particles to which Lp-PLA$_2$ associates preferentially (3, 20, 21). In turn, the CET process is stimulated by negatively charged cholesteryl ester acceptor lipoproteins, although a delicate balance in the concentration and structure of various long chain nonesterified fatty acids seems to be required for maximal stimulation of CET (16, 18, 40). Additionally, a higher affinity of CETP for oxidized LDL has been demonstrated *in vitro*, which may be attributable to the generation of negatively charged lipids during LDL oxidation (16, 41). Association of CETP with negatively charged LDL may affect the CET process by targeting transfer of HDL-derived cholesteryl esters more preferentially toward LDL (9, 16). Thus, it is likely that the composition of apo B-containing lipoproteins, including their charge characteristics, affect both plasma CET and their association with Lp-PLA$_2$ in the same direction. It seems plausible that the independent relationship of plasma CET with Lp-PLA$_2$ is not merely associative, but could imply a causal role of Lp-PLA$_2$ in CET regulation or vice versa. It can be anticipated that generation of negatively charged oxidized nonesterified fatty acids elicited by Lp-PLA$_2$ may increase directly the electronegativity of apo B-containing lipoproteins (3) and hence stimulate the CET process. In this respect, it is noteworthy that Lp-PLA$_2$ is able to hydrolyze long acyl chain oxidized phospholipids, thereby generating long acyl chain oxidized nonesterified fatty acids and fatty acid hydroperoxides (3). Conversely, the CET process contributes to the formation of (electronegative) small, dense LDL (8, 9, 10), which may preferentially bind Lp-PLA$_2$ (3, 21). Furthermore, CETP action could also promote generation of oxidized LDL *in vivo* (42). Accordingly, resistance of LDL particles to oxidative modification has been observed after immunological inhibition of CETP *in vitro* (43). We therefore hypothesize that the relationship of plasma CET with Lp-PLA$_2$, as shown in this report, may represent implication of Lp-PLA$_2$-mediated generation of oxidized nonesterified fatty acids in the CET process. Alternatively, this relationship could imply that the CET process could contribute to generation of oxidized lipids rendering apo B-containing lipoprotein (subfractions) more electronegative, which may enhance Lp-PLA$_2$ association with LDL particles.

Both possibilities can be anticipated to promote the atherogenicity of plasma lipoproteins. If Lp-PLA$_2$ enhances the CET process, then the contribution of circulating Lp-PLA$_2$ to atherosclerosis susceptibility could in part be explained by a hitherto unappreciated contribution to an unfavorable distribution of cholesterol between HDL and apo B-containing lipoproteins (9, 10, 12, 17). Conversely, if plasma CET stimulates binding of Lp-PLA$_2$ to apo B-containing lipoproteins, then this process may

aggravate the prooxidative and proinflammatory potential of these lipoproteins, including small, dense LDL (3, 44). Obviously, the precise mechanisms responsible for the relationship of plasma CET with Lp-PLA_2 should be determined in future experiments. Clearly, the present findings provide a rationale to test whether pharmacological inhibition of Lp-PLA_2 (45, 46) will selectively reduce the plasma CET process. Furthermore, it may be relevant to know whether CETP inhibitors, which are anticipated to increase LDL size (12–14), affect Lp-PLA_2 binding to LDL and its prooxidant activity.

Early studies have demonstrated that EST in incubated plasma is stimulated by cholesteryl ester acceptor lipoproteins (24). This mechanism explains why the plasma triglyceride concentration is a determinant of EST, besides an effect of the LCAT activity level *per se* (25, 26). Our study entirely agrees with this finding (25) and reiterates previously documented increases in plasma EST (22) and LCAT activity (15, 47) in MetS and dyslipidemic subjects. The observation that CET can become rate limiting for EST (24) most likely also underlies the strong relationship of EST with CET (25, 26). In this respect, it is noteworthy that there was no positive association of plasma EST with Lp-PLA_2. Although HDL size and lipid compositional characteristics affect their binding affinity for CETP (48), there are to our knowledge no reports that demonstrate preferential binding of CETP to electronegative HDL particles. Moreover, only a minor proportion of Lp-PLA_2 is associated with HDL (3), even in dyslipidemic and diabetic plasma where Lp-PLA_2 may be redistributed from apo B-containing lipoproteins toward HDL (4). Thus, the present report suggests that effects of Lp-PLA2 on components of the reverse cholesterol transport pathway are confined to CET.

In conclusion, this study has revealed robust independent relationships of plasma CET, but not EST, with the Lp-PLA_2 level in subjects with and without the MetS. This finding raises the possibility that Lp-PLA_2 may specifically affect the CET process, or alternatively that CETP inhibition could affect Lp-PLA_2 activity.

Acknowledgments

The analytical help of L. D. Dikkeschei, Ph.D. (Isala Klinieken, Zwolle, The Netherlands), for measurement of plasma lipids and of G. M. Dallinga-Thie, Ph.D. (Laboratory of Experimental Vascular Medicine, University of Amsterdam, The Netherlands), for CETP mass measurement is much appreciated. The reagents for Lp-PLA_2 mass analysis are kindly donated by diaDexus Inc. (San Francisco, CA).

Address all correspondence and requests for reprints to: R. P. F. Dullaart, M.D., Ph.D., Department of Endocrinology, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, Groningen 9700 RB, The Netherlands. E-mail: r.p.f.dullaart@int.umcg.nl.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Caslake MJ, Packard CJ** 2005 Lipoprotein-associated phospholipase A_2 as a biomarker for coronary disease and stroke. Nat Clin Pract Cardiovasc Med 2:529–535
2. **Wilensky RL, Macphee CH** 2009 Lipoprotein-associated phospholipase A(2) and atherosclerosis. Curr Opin Lipidol 20:415–420
3. **Tellis CC, Tselepis AD** 2009 The role of lipoprotein-associated phospholipase A_2 in atherosclerosis may depend on its lipoprotein carrier in plasma. Biochim Biophys Acta 1791:327–338
4. **Kujiraoka T, Iwasaki T, Ishihara M, Ito M, Nagano M, Kawaguchi A, Takahashi S, Ishi J, Tsuji M, Egashira T, Stepanova IP, Miller NE, Hattori H** 2003 Altered distribution of plasma PAF-AH between HDLs and other lipoproteins in hyperlipidemia and diabetes mellitus. J Lipid Res 44:2006–2014
5. **Iwase M, Sonoki K, Sasaki N, Ohdo S, Higuchi S, Hattori H, Iida M** 2008 Lysophosphatidylcholine contents in plasma LDL in patients with type 2 diabetes mellitus: relation with lipoprotein-associated phospholipase A2 and effects of simvastatin treatment. Atherosclerosis 196:931–936
6. **Sonoki K, Iwase M, Sasaki N, Ohdo S, Higuchi S, Matsuyama N, Iida M** 2009 Relations of lysophosphatidylcholine in low-density lipoprotein with serum lipoprotein-associated phospholipase A2, paraoxonase and homocysteine thiolactonase activities in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract 86:117–123
7. **Lp-PLA(2) Studies Collaboration, Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S, Ballantyne C, Cannon CP, Criqui M, Cushman M, Hofman A, Packard C, Thompson SG, Collins R, Danesh J** 2010 Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. Lancet 375:1536–1544
8. **Tall AR** 1993 Plasma cholesteryl ester transfer protein. J Lipid Res 34:1255–1274
9. **Borggreve SE, De Vries R, Dullaart RP** 2003 Alterations in high-density lipoprotein metabolism and reverse cholesterol transport in insulin resistance and type 2 diabetes mellitus: role of lipolytic enzymes, lecithin:cholesterol acyltransferase and lipid transfer proteins. Eur J Clin Invest 33:1051–1069
10. **Dullaart RP, Dallinga-Thie GM, Wolffenbuttel BH, van Tol A** 2007 CETP inhibition in cardiovascular risk management: a critical appraisal. Eur J Clin Invest 37:90–98
11. **Chapman MJ, Le Goff W, Guerin M, Kontush A** 2010 Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with statins, fibrates, niacin, and cholesteryl ester transfer protein inhibitors. Eur Heart J 31:149–164
12. **Kappelle PJ, van Tol A, Wolffenbuttel BH, Dullaart RP** 14 July 2010 Cholesteryl ester transfer protein inhibition in cardiovascular risk management: ongoing trials will end the confusion. Cardiovasc Ther doi: 10.1111/j.1755-5922.2010.00201.x
13. **Krishna R, Anderson MS, Bergman AJ, Jin B, Fallon M, Cote J, Rosko K, Chavez-Eng C, Lutz R, Bloomfield DM, Gutierrez M, Doherty J, Bieberdorf F, Chodakewitz J, Gottesdiener KM, Wagner JA** 2007 Effect of the cholesteryl ester transfer protein inhibitor, anacetrapib, on lipoproteins in patients with dyslipidaemia and on 24-h ambulatory blood pressure in healthy individuals: two double-blind, randomised placebo-controlled phase I studies. Lancet 370:1907–1914
14. **Schwartz GG, Olsson AG, Ballantyne CM, Barter PJ, Holme IM, Kallend D, Leiter LA, Leitersdorf E, McMurray JJ, Shah PK, Tardif JC, Chaitman BR, Duttlinger-Maddux R, Mathieson J; dal-OUTCOMES Committees and Investigators** 2009 Rationale and

design of the dal-OUTCOMES trial: efficacy and safety of dalcetrapib in patients with recent acute coronary syndrome. Am Heart J 158:896–901.e3

15. **Dullaart RP, Groener JE, Erkelens DW** 1987 Effect of the composition of very low and low density lipoproteins on the rate of cholesteryl ester transfer from high density lipoproteins in man, studied in vitro. Eur J Clin Invest 17:241–248
16. **Lagrost L** 1994 Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. Biochim Biophys Acta 1215:209–236
17. **de Vries R, Perton FG, Dallinga-Thie GM, van Roon AM, Wolffenbuttel BH, van Tol A, Dullaart RP** 2005 Plasma cholesteryl ester transfer is a determinant of intima-media thickness in type 2 diabetic and nondiabetic subjects: role of CETP and triglycerides. Diabetes 54:3554–3559
18. **Nishida HI, Arai H, Nishida T** 1993 Cholesterol ester transfer mediated by lipid transfer protein as influenced by changes in the charge characteristics of plasma lipoproteins. J Biol Chem 268: 16352–16360
19. **Lagrost L, Florentin E, Guyard-Dangremont V, Athias A, Gandjini H, Lallemant C, Gambert P** 1995 Evidence for nonesterified fatty acids as modulators of neutral lipid transfers in normolipidemic human plasma. Arterioscler Thromb Vasc Biol 15:1388–1396
20. **Benítez S, Sánchez-Quesada JL, Ribas V, Jorba O, Blanco-Vaca F, González-Sastre F, Ordóñez-Llanos J** 2003 Platelet-activating factor acetylhydrolase is mainly associated with electronegative low-density lipoprotein subfraction. Circulation 108:92–96
21. **Gaubatz JW, Gillard BK, Massey JB, Hoogeveen RC, Huang M, Lloyd EE, Raya JL, Yang CY, Pownall HJ** 2007 Dynamics of dense electronegative low density lipoproteins and their preferential association with lipoprotein phospholipase A(2). J Lipid Res 48:348–357
22. **Dullaart RP, Groen AK, Dallinga-Thie GM, de Vries R, Sluiter WJ, van Tol A** 2008 Fibroblast cholesterol efflux to plasma from metabolic syndrome subjects is not defective despite low HDL cholesterol. Eur J Endocrinol 158:53–60
23. **Riemens SC, van Tol A, Scheek LM, Dullaart RP** 2001 Plasma cholesteryl ester transfer and hepatic lipase activity are related to high-density lipoprotein cholesterol in association with insulin resistance in type 2 diabetic and non-diabetic subjects. Scand J Clin Lab Invest 61:1–9
24. **Fielding CJ, Fielding PE** 1981 Regulation of human plasma lecithin: cholesterol acyltransferase activity by lipoprotein acceptor cholesteryl ester content. J Biol Chem 256:2102–2104
25. **Riemens S, van Tol A, Sluiter W, Dullaart RP** 1998 Elevated plasma cholesteryl ester transfer in NIDDM: relationships with apolipoprotein B-containing lipoproteins and phospholipid transfer protein. Atherosclerosis 140:71–79
26. **Dullaart RP, Riemens SC, Scheek LM, Van Tol A** 1999 Insulin decreases plasma cholesteryl ester transfer but not cholesterol esterification in healthy subjects as well as in normotriglyceridaemic patients with type 2 diabetes. Eur J Clin Invest 29:663–671
27. **Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith Jr SC, Spertus JA, Costa F** 2005 Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 112:2735–2752
28. **Channon KM, Clegg RJ, Bhatnagar D, Ishola M, Arrol S, Durrington PN** 1990 Investigation of lipid transfer in human serum leading to the development of an isotopic method for the determination of endogenous cholesterol esterification and transfer. Atherosclerosis 80:217–226
29. **Sutherland WH, Walker RJ, Lewis-Barned NJ, Pratt H, Tillman HC** 1994 Plasma cholesteryl ester transfer in patients with non-insulin dependent diabetes mellitus. Clin Chim Acta 231:29–38
30. **van Venrooij FV, Stolk RP, Banga JD, Sijmonsma TP, van Tol A, Erkelens DW, Dallinga-Thie GM; DALI Study Group** 2003 Common cholesteryl ester transfer protein gene polymorphisms and the effect of atorvastatin therapy in type 2 diabetes. Diabetes Care 26: 1216–1223
31. **Dullaart RP, De Vries R, Scheek L, Borggreve SE, Van Gent T, Dallinga-Thie GM, Ito M, Nagano M, Sluiter WJ, Hattori H, Van Tol A** 2004 Type 2 diabetes mellitus is associated with differential effects on plasma cholesteryl ester transfer protein and phospholipid transfer protein activities and concentrations. Scand J Clin Lab Invest 64:205–215
32. **Glomset JA, Wright JL** 1964 Some properties of a cholesterol esterifying enzyme in human plasma. Biochim Biophys Acta 89:266–276
33. **Dullaart RP, Sluiter WJ, Dikkeschei LD, Hoogenberg K, Van Tol A** 1994 Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism. Eur J Clin Invest 24:188–194
34. **Dullaart RP, Perton F, Kappelle PJ, de Vries R, Sluiter WJ, van Tol A** 2010 Plasma lecithin:cholesterol acyltransferase activity modifies the inverse relationship of C-reactive protein with HDL cholesterol in nondiabetic men. Biochim Biophys Acta 1801:84–88
35. **Albers JJ, Chen CH, Adolphson JL** 1981 Lecithin:cholesterol acyltransferase (LCAT) mass; its relationship to LCAT activity and cholesterol esterification rate. J Lipid Res 22:1206–1213
36. **Florén CH, Chen CH, Franzén J, Albers JJ** 1987 Lecithin:cholesterol acyltransferase in liver disease. Scand J Clin Lab invest 47:613–617
37. **Mann CJ, Yen FT, Grant AM, Bihain BE** 1991 Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. J Clin Invest 88:2059–2066
38. **Persson M, Hedblad B, Nelson JJ, Berglund G** 2007 Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. Arterioscler Thromb Vasc Biol 27:1411–1416
39. **Noto H, Chitkara P, Raskin P** 2006 The role of lipoprotein-associated phospholipase A(2) in the metabolic syndrome and diabetes. J Diabetes Complications 20:343–348
40. **Lagrost L, Barter PJ** 1992 Cholesteryl ester transfer protein promotes the association of HDL apolipoproteins A-I and A-II with LDL: potentiation by oleic acid. Biochim Biophys Acta 1127:255–262
41. **Zawadzki Z, Milne RW, Marcel YL** 1991 Cu^{2+}-mediated oxidation of dialyzed plasma: effects on low and high density lipoproteins and cholesteryl ester transfer protein. J Lipid Res 32:243–250
42. **Wang J, Gu Q, Li K, Zhang C** 2007 CETP and oxidized LDL levels increase in dyslipidemic subjects. Clin Biochem 40:995–999
43. **Sugano M, Sawada S, Tsuchida K, Makino N, Kamada M** 2000 Low density lipoproteins develop resistance to oxidative modification due to inhibition of cholesteryl ester transfer protein by a monoclonal antibody. J Lipid Res 41:126–133
44. **Sánchez-Quesada JL, Benítez S, Pérez A, Wagner AM, Rigla M, Carreras G, Vila L, Camacho M, Arcelus R, Ordóñez-Llanos J** 2005 The inflammatory properties of electronegative low-density lipoprotein from type 1 diabetic patients are related to increased platelet-activating factor acetylhydrolase activity. Diabetologia 48:2162–2169
45. **Chauffe RJ, Wilensky RL, Mohler 3rd ER** 2010 Recent developments with lipoprotein-associated phospholipase A2 inhibitors. Curr Atheroscler Rep 12:43–47
46. **White H** 2010 Why inhibition of lipoprotein-associated phospholipase A_2 has the potential to improve patient outcomes. Curr Opin Cardiol 25:299–301
47. **Dullaart RP, Perton F, van der Klauw MM, Hillege HL, Sluiter WJ; PREVEND Study Group** 2010 High plasma lecithin:cholesterol acyltransferase activity does not predict low incidence of cardiovascular events: possible attenuation of cardioprotection associated with high HDL cholesterol. Atherosclerosis 208:537–542
48. **Bruce C, Davidson WS, Kussie P, Lund-Katz S, Phillips MC, Ghosh R, Tall AR** 1995 Molecular determinants of plasma cholesteryl ester transfer protein binding to high density lipoproteins. J Biol Chem 270:11532–11542